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Larsen, Martin Hage; Thorn, Aske N.; Skov, Christian; Aarestrup, Kim

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Effects of passive integrated transponder tags on survival and growth of juvenile Atlantic salmon *Salmo salar*

Martin H Larsen*, Aske N Thorn, Christian Skov and Kim Aarestrup

Abstract

Background: A laboratory experiment was conducted to assess the potential impacts of surgically implanted 23 and 32 mm passive integrated transponder (PIT) tags on survival, growth, and body condition of juvenile Atlantic salmon *Salmo salar*. Rate of tag retention and healing of the tagging incision were also evaluated. Atlantic salmon of three different size classes (I: 80 to 99 mm fork length (FL), II: 100 to 119 mm FL, III: 120 to 135 mm FL) were allocated to each of five experimental treatment groups: control, sham-operated (surgery without PIT-tag implantation), 23 mm PIT-tag implantation with and without suture closure of the incision, and 32 mm PIT-tag implantation without suture closure.

Results: Over the 35-day experiment, mortality occurred only among fish tagged with 32 mm PIT tags (14%) and all fish larger than 103 mm FL survived. Non-sutured Atlantic salmon between 80 and 99 mm FL implanted with 23 mm PIT tags had a significantly lower mean specific growth rate of mass compared with untagged (control and sham-operated) and sutured conspecifics. However, no significant difference in growth was found between untagged fish and 23 mm PIT-tagged fish 100 to 135 mm FL. Implantation of 32 mm PIT tags decreased growth in all size classes. Regardless of size class, body condition of the fish was not affected by PIT tagging. Retention rates of 23 mm PIT tags with and without suture closure were 100% and 97%, respectively; retention of 32 mm PIT tags without suture closure was 69%. At the end of the experiment, tagging incisions without suture closure were generally well-healed. Fungal infection and inflammation around the incision site occurred only when suture was used, in 46% of size class I, 21% of size class II and 38% of size class III.

Conclusions: Although suture closure of the incision following 23 mm PIT-tag implantation had a positive impact on growth of fish smaller than 100 mm FL, we advise against the use of sutures due to high rates of fungal infection around the incision site. Hence, results suggest that surgical implantation of 23 mm PIT tags without suture closure of the incision is a feasible method for marking juvenile Atlantic salmon 100 to 135 mm FL. Further, we caution researchers about the use of 32 mm PIT tags in juvenile Atlantic salmon 80 to 135 mm FL due to high rate of tag rejection and reduced survival and growth.

Keywords: Biotelemetry, PIT tagging, Salmonids, Surgery, Tagging effect

Background

Over the past decades, passive integrated transponder (PIT) tags have proven to be a powerful tool for monitoring migration, growth, survival, and spatio-temporal distribution of various fish species [1-6]. PIT tags are advantageous due to their longevity, small size, and ability to equip individuals with a unique identification code. Furthermore, PIT tags provide a cost-effective and easy internal tagging technique for gathering information about fish ecology. Currently, commercially available PIT tags typically vary in length from 11 to 32 mm. Smaller tags generally have a lower detection range than larger ones when energized by external antennae via an electromagnetic signal [7]. As a consequence, the use of smaller transponder tags (for example, 11 to 12 mm) has largely been restricted to laboratory applications and field studies in systems with water depths less than 40 cm [8,9]. Larger tag sizes, such as 23 or 32 mm, offer a detection range up to 100 cm, extending their applicability for certain

* Correspondence: mhala@aqua.dtu.dk
National Institute of Aquatic Resources, Freshwater Fisheries, Technical University of Denmark, Vejlsøvej 39, Silkeborg DK-8600, Denmark

telemetry applications [10,11]. However, because intracoelomic implantation of PIT tags may adversely affect fish [12,13], scientists must balance the often conflicting needs of high detection range and low tag burden (tag size relative to fish body size), especially in telemetry studies involving smaller-bodied fish.

A growing number of studies have addressed the adverse effects of PIT tagging on smaller fish and/or various life stages [12-18]. In juvenile salmonids, the impact of smaller PIT tags (11 to 12 mm) on growth and mortality have generally been negligible (for example, brown trout *Salmo trutta* [3]; Atlantic salmon *Salmo salar* [19]) and tag retention rates are usually high (that is, 97% to 100% [3,19,20], see [21] for an exception). The efficacy of 23 mm PIT tags has also been tested among different species of juvenile salmonids (Atlantic salmon *S. salar* [10,22]; coho salmon *Oncorhynchus kisutch* [20]; steelhead *O. mykiss* [14,20,23]). For instance, Bateman and Gresswell [14] examined growth and survival of steelhead (73 to 97 mm FL) following intracoelomic surgical implantation of 23 mm tags. The authors found transient reduction in growth and higher mortality among tagged steelhead compared to control and sham-operated fish (that is, fish that received surgery but no PIT tag). Using the same-sized tag and surgical technique, Roussel *et al.* [10] used PIT technology to study diel movements and habitat use by Atlantic salmon parr 64 to 94 mm FL. However, no control or sham-operated group was included in the study, sample size was small (n = 33), and growth was not evaluated as an endpoint. Thus, there is a need for additional evaluation and clarification of whether and to what extent 23 mm PIT tags influence survival and growth of juvenile Atlantic salmon. Moreover, to our knowledge, no studies have previously tested the efficacy of 32 mm PIT tags in smaller fish. Hence, the aim of the present study was to elucidate the potential effects of surgically implanted 23 and 32 mm PIT tags on survival, growth, and body condition of juvenile Atlantic salmon. Incision healing with and without suture closure and rate of tag retention were also assessed.

Results

Mortality

While there were no mortalities for control, sham-operated, and 23 mm PIT-tagged fish with and without suture closure of the tagging incision, 10 fish with 32 mm PIT tags died during the laboratory experiment. This mortality rate (14%) was statistically different from that of the other treatments (Chi-square test, $\chi^2_4 = 41.143$, $P < 0.0001$). The majority of mortalities (90%) resulting from 32 mm PIT tags occurred within 11 days after tagging (the last fish died 21 days after tagging) but all Atlantic salmon larger than 103 mm FL survived. However, there were no mortalities during surgery or the recovery period immediately after surgery and tag insertion. Average tag-to-body mass ratio of dead and surviving fish tagged with 32 mm PIT tags were 11.1% and 5.0%, respectively. External and internal examination of the dead fish revealed no abnormalities (for example, accidental cuts or punctures to internal organs from the scalpel, tissue inflammation, or infection around incision).

Growth rate

At the end of the 35-day experiment, the mean specific growth rate (SGR; g day^{-1}) differed significantly among treatments within each of the three size classes (one-way analysis of variance (ANOVA), degrees of freedom = 96 to 114, $F \geq 4.204$, $P \leq 0.004$; Figure 1). For size class I (80 to 99 mm FL), post hoc comparisons showed that fish tagged with 23 and 32 mm PIT tags without suture closure of the incision had significantly lower mean SGR than control, sham-operated, and 23 mm PIT-tagged individuals with suture closure (Tukey honestly significant difference (HSD) test, $P \leq 0.041$; Figure 1). In fact, the mean SGR was negative for non-sutured fish implanted with 23 and 32 mm PIT tags at the end of the study. For size classes II (100 to 119 mm FL) and III (120 to 135 mm FL), only growth of fish implanted with 32 mm tags differed significantly from that of the other treatments (Tukey HSD test, all $P \leq 0.001$; Figure 1). No differences in mean SGR was found between control and sham-operated fish within the three size classes (Tukey HSD test, all $P \geq 0.990$).

Body condition

Including all size classes, a full-factorial analysis of covariance on the final body mass of the Atlantic salmon with treatment as fixed factor and final length as covariate demonstrated no interaction between treatments and final length ($F_{4,\ 322} = 1.961$, $P = 0.100$). A subsequent additive model including the interaction term in the error variance showed a significant effect of final length ($F_{1,\ 326} = 31587.767$, $P < 0.0001$), but no effect of treatment ($F_{4,\ 326} = 0.714$, $P = 0.583$). It seems, therefore, that the body condition of the Atlantic salmon was not affected by surgical implantation of 23 and 32 mm PIT tags during the study.

Tag retention and incision healing

The tag retention rates without suture closure were 97% and 69% for the 23 and 32 mm PIT-tagged treatment group, respectively, and no tag loss was recorded when sutures were used. Regardless of tag size, the majority of tag losses (88%) occurred within the first 16 days of the experiment and no 23 mm PIT tags were lost after day 13. Fish that lost their 32 mm PIT tag were on average significantly smaller (96 ±1.88 mm FL, n = 22) than those that retained their tag (114 ±2.98 mm FL, n = 50) (t-test, $t_{70} = 4.863$, $P < 0.0001$). The two fish that rejected their 23 mm PIT tag measured 82 and 86 mm FL at tagging.

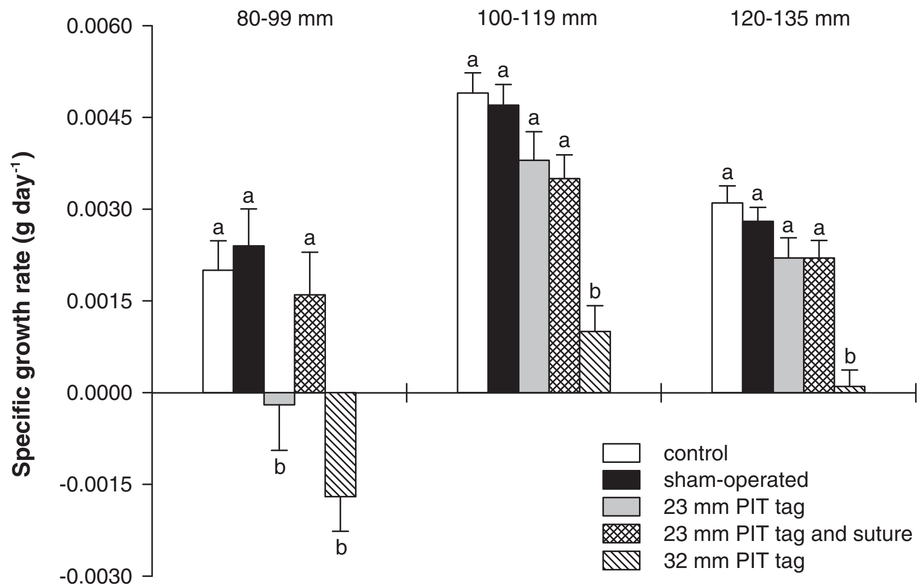

Figure 1 Mean specific growth rate of Atlantic salmon *Salmo salar* among treatment groups in three different size classes. Data calculated as mean specific growth rate (g day^{-1}) ± standard error. Sample size for size classes I (80 to 99 mm FL), II (100 to 119 mm FL) and III (120 to 135 mm FL) were as follows: control (n = 24, 24, 24), sham-operated (n = 24, 24, 24), 23 mm PIT tag (n = 22, 24, 24), 23 mm PIT tag and suture (n = 24, 24, 24), 32 mm PIT tag (n = 7, 16, 23). Differences in sample size are due to mortality and loss of PIT tags. Within each size class, bars not sharing the same letter are significantly different at P <0.05 (Tukey honestly significant difference). PIT, passive integrated transponder.

The tagging incisions without suture closure (n = 216) were generally well healed and no signs of tissue inflammation or infection were observed. By contrast, 31% (n = 22 of 72) of sutured incisions exhibited mild to moderate fungal infection around the incision and suture insertion site. Furthermore, a few sutured incisions (4%) were partially open and highly inflamed and infected, resulting in an overall infection rate of 35%. Although the prevalence of fungal infection was particularly high for size class I (46%), no significant difference in the infection rate was observed between the three size classes (Chi-square test, $\chi^2_2 = 2.288$, $P = 0.319$). The prevalence of fungal infection was 21% and 38% for size class II and III, respectively. It is also noteworthy that all fish that lost the suture (15% shed rate) during the experiment had well healed incisions free of infections.

Discussion

Mortality

During the 35-day laboratory experiment, 10 fish tagged with 32 mm PIT tags died (14%), whereas no mortalities occurred among control, sham-operated or 23 mm PIT-tagged fish with and without suture closure of the incision. The majority of mortality (80%) occurred in fish 80 to 84 mm FL. This is in agreement with results from other studies that typically report higher mortalities in smaller individuals following tagging [18,21,24,25]. For example, Acolas *et al.* [21] demonstrated that increases in length of juvenile brown trout (41 to 70 mm FL) significantly enhanced the probability of survival after implantation of 11.5 mm PIT tags. Similar results have been reported for Atlantic salmon parr (60 to 69 mm FL) using 11.5 mm PIT tags [24].

The exact cause of death in 32 mm PIT-tagged fish is somewhat unclear. Necropsy revealed no evidence of cuts or punctures to internal organs from the scalpel and epidermis infection was not observed around incisions. However, as the peritoneal cavity of the smaller fish was very limited in its capacity to accommodate a 32 mm tag, it is possible that the tag interfered with vital body organs. Furthermore, direct observations during the study revealed that implanted 32 mm PIT tags influenced buoyancy of tagged fish and resulted in unnatural swimming behavior. This may suggest that the swim bladder was not able to compensate for the additional tag weight. Because a substantial part of the body cavity was occupied by the 32 mm PIT tag, it is also possible that there was not enough room for full expansion of the swim bladder.

In a previous laboratory study, Roussel *et al.* [10] reported a mortality rate of 21% for Atlantic salmon parr (<84 mm FL) following surgical implantation of 23 mm PIT tags. However, the authors did not compare the survival of tagged fish with a control group and sample size was small (n = 33). A 99% survival rate for Atlantic salmon (>90 mm FL) tagged with 23 mm PIT tags was reported by Zydlewski *et al.* [22]. Hence, our results and those of

Zydlewski *et al.* [22] suggest that 23 mm PIT tags have no measurable impact on survival of juvenile Atlantic salmon 80 to 135 mm FL.

Growth and body condition

While non-sutured fish between 80 and 99 mm FL implanted with 23 mm PIT tags had a lower mean SGR compared to control and sham-operated individuals, no significant difference was detected in fish with sutured incisions. Although the reason remains unknown, this finding suggests that incision suturing following tagging may have a positive impact on the mean SGR of Atlantic salmon 80 to 99 mm FL. It should be noted, however, that the average growth of sutured fish between 80 and 99 mm FL was still somewhat lower (16%) than growth of the control group and 46% of the fish exhibited fungal infection around the incision site. Hence, long-term studies investigating the effect of fungal infection on growth and survival are needed before recommending the use of suture in Atlantic salmon 80 to 99 mm FL. The mean SGR for fish between 100 and 135 mm FL was not significantly affected by 23 mm PIT-tag implantation with and without suture closure of the incision, although the mean SGR was up to 30% lower compared to the control group. However, 32 mm PIT tags significantly affected mean SGR of all size classes. Mean SGR was not significantly different between control and sham-operated fish within all size classes, implying that the surgical procedures had no effects on mean SGR *per se*, which is consistent with earlier findings [14,26,27]. Therefore, the presence of the tag is evidently responsible for the decreased growth, perhaps due to increased energy expenditure of carrying the tag or physiological adjustments needed to accommodate it.

Several other studies have reported depressed growth after tagging, but there is much variation in the extent and duration of this impact, depending on factors such as species, size, and environmental conditions [12,14,18,21,24]. Bateman and Gresswell [14] showed decreased growth in juvenile steelhead (73 to 97 mm FL) during the first 20 days after surgical implantation of a 23 mm PIT tag. However, this was compensated for 30 days after tagging by increased growth of the tagged fish. Similar results have been found for juvenile brown trout (41 to 70 mm FL) and Atlantic salmon (60 to 69 mm FL) up to 60 days after implantation with 11.5 mm tags [21,24]. Tatara [13] demonstrated that the probability of experiencing positive growth in steelhead parr (45 to 96 mm FL) implanted with 12 mm PIT tags was size-dependent, and it was concluded that length at implantation should be above 74 mm FL to avoid negative growth. While we were not able to identify the length at tagging that would prevent negative effect of 32 mm tags on growth of Atlantic salmon, results indicate that implantation of 23 mm PIT tags has no significant effect on growth of fish 100 to 135 mm FL. As the duration of the study was short (35 days), it remains unknown whether the somewhat lower growth of the PIT-tagged fish would eventually be accounted for through compensatory growth. Moreover, although the condition of the fish in terms of length-mass relationships was similar among treatments at the end of the experiment, slower growth rates due to tagging could have long-term repercussions on body condition. Additional studies are needed to examine the long-term impact of 23 and 32 mm PIT tags on growth and body condition of Atlantic salmon.

Tag retention and incision healing

Tag retention of 23 mm PIT tags was high (97%) when the tagging incisions were left to heal without suture closure and no tag loss occurred when sutures were used. The two fish that lost their 23 mm tags were both below 90 mm FL. These results are in accordance with those of Zydlewski *et al.* [22], reporting a 23 mm PIT tag retention rate of 99% for Atlantic salmon (>90 mm FL). Roussel *et al.* [10], however, found a tag loss rate of 15% for Atlantic salmon parr (64 to 94 mm FL) implanted with the same-sized tag, but 100% retention when suture was used to close the incision. Suturing may therefore considerably lower the rate of tag loss through the incision in smaller fish [10,12,25]. Additionally, suture closure has been shown to enhance the rate of incision healing and reduce the risk of infection and epidermis inflammation for certain fish species [12]. In the current investigation, however, tissue inflammation and fungal infection were only observed when sutures were used and non-sutured incisions were generally well-healed. This observation and low 23 mm PIT tag rejection rate without suture closure, suggest that PIT tag incisions should not be closed with suture in juvenile Atlantic salmon 80 to 135 mm FL. At the same time, suturing the incision increases handling time and risk of puncturing internal organs [12]. It should be emphasized, that tagging incisions should be made small enough (3 to 4 mm) so that the 23 mm transponder tag cannot easily work its way out of the wound again. Finally, tissue adhesives (usually cyanoacrylate) are occasionally used as an alternative to sutures, as it might be a faster and less intrusive way of closing incisions [28,29]. However, because incisions closed with adhesives sometimes reopen and result in tissue inflammation, the efficacy of this closing technique may be questioned [25,29,30]. Clearly there is a need for additional work focused on comparing multiple incision closure methods in smaller fish.

After 35 days, 31% of the Atlantic salmon had lost their 32 mm transponder tag. Such a high tag loss rate is greater than the accepted value for juvenile salmonids, which is usually below 10% [3,14,19,20,22,23]. The majority of 32 mm tag loss occurred among smaller individuals and was most likely caused by limited body cavity capacity. In a narrow peritoneal cavity, pressure against the tag could cause it to be pushed through the tagging incision. Other researchers have also reported a relationship between length at tagging and rate of tag retention, with larger individuals typically having the highest rate of tag retention [21]. Most tag losses occurred within the first half of the experiment and tag loss presumably decreased as tagging incisions began to heal. Thus, additional tag losses related to tagging are believed to be negligible 35 days after surgery [14,25].

Conclusions

Surgical implantation of 23 mm PIT tags into the body cavity of juvenile Atlantic salmon (80 to 135 mm FL) did not affect survival or body condition and tag retention was high with and without absorbable suture closure of the tagging incisions. Although suture closure of the incision had a positive effect on growth of the smaller 23 mm PIT-tagged fish (80 to 99 mm FL), 46% of the incisions were infected by fungus. Fungal infections were also observed in sutured fish 100 to 119 mm FL (21%) and 120 to 135 mm FL (38%). By contrast, all non-sutured incisions were generally well-healed and no signs of epidermis inflammation or fungal infection were observed. Hence, at this point, we caution researchers about the use of sutures in juvenile Atlantic salmon 80 to 135 mm FL. Further studies are needed to examine the long-term effects of fungal infection around the incision and suture site on growth and mortality of juvenile Atlantic salmon.

When leaving the incision non-sutured, results indicate that 23 mm PIT tags have no adverse effect on growth of Atlantic salmon 100 to 135 mm FL. We conclude that intracoelomic implantation of 23 mm PIT tags without suture closure is a useful method for individual marking of Atlantic salmon 100 to 135 mm FL. However, in studies where growth is not a parameter of interest, 23 mm PIT tags may be useful in Atlantic salmon as small as 80 mm FL. Finally, we recommend that 32 mm PIT tags should not be used use in juvenile Atlantic salmon (80 to 135 mm FL) due to high mortality, high tag loss rate, and reduced growth. We advocate studies on larger Atlantic salmon to establish a suitable size limit for using these tags.

It is difficult to anticipate whether the results from the present laboratory study will be applicable in natural systems. Fish in a hatchery are not exposed to the same stressors (predation, food, density, pathogens) that fish in the wild experience. As such, future tagging studies would benefit greatly from combining both laboratory and field experiments. Moreover, it is possible that the effect of tagging hatchery-raised fish may be different from those of wild fish. Nevertheless, the results of the present investigation should be useful for managers and scientists in monitoring the biology and ecology of Atlantic salmon.

Methods

Experimental fish

Fish used in the experiment were hatchery-reared offspring of wild Atlantic salmon caught by electrofishing in River Storå, Denmark. Fertilized eggs were incubated in egg trays and hatched in late March 2011 at the Danish Centre for Wild Salmon (DCV) in Randers, Denmark. After hatching, the alevins were maintained in the hatching trays until the yolk sac was completely absorbed. Fish were then transferred to flow-through tanks for exogenous feeding and kept under ambient photoperiod and temperature (4°C to 17°C) conditions. The Atlantic salmon were fed daily with commercial trout pellets equivalent to 1.5% to 4% of body mass. All study fish were handled in accordance to the guidelines described in permission (2012-DY-2934-00007) from the Danish Experimental Animal Committee.

Experimental protocol

The laboratory experiment was conducted at the hatchery facilities at DCV from 21 December 2011 to 25 January 2012. A total of 360 Atlantic salmon were divided into three different size classes (n = 120 per size class): I: 80 to 99 mm FL, II: 100 to 119 mm FL, III: 120 to 135 mm FL. Within each size class, fish were randomly assigned to one of five treatment groups: control, sham-operated, 23 mm PIT tag, 23 mm PIT tag and suture, and 32 mm PIT tag. This resulted in 24 fish from each size class per treatment. These fish were evenly and haphazardly distributed among six experimental tanks. Hence, each tank contained fish from all five treatments and each treatment group consisted of four fish from each size class per tank. The net result was 60 fish in each tank (that is, 12 fish per treatment group). Within size classes, there were no significant differences in length and mass among the treatment groups at the beginning of the experiment (one-way ANOVA, $F_{4,115} \leq 2.236$, all $P \geq 0.069$).

Treatment fish were placed in an anesthetic bath (benzocaine 20 mg L^{-1}) until the opercular rate became slow and irregular (4 to 5 min). Once unresponsive, the fork length and body mass were measured to the nearest 1 mm and 0.1 g, respectively. Sham-operated fish received a 3 to 4 mm ventrolateral incision, 5 to 7 mm anterior to the muscle bed of the pelvic fins on the left side of the body. Atlantic salmon in the 23 mm PIT-tagged group were treated similarly except a uniquely coded 23 mm PIT tag (RI-TRP-RRHP, half duplex, 134 kHz, diameter 3.85 mm and weight 0.6 g in air; Texas Instruments, Plano, Texas, USA) was inserted into the peritoneal cavity through the incision. For the 23 mm PIT-tagged fish with suture closure treatment, incisions were closed with one stitch of absorbable suture (Vicryl 5–0 FS-2; Ethicon, Piscataway, NJ, USA) tied with a single surgeons knot. Fish in the 32 mm PIT-tagged treatment group were subjected to a 3 to 4 mm ventrolateral incision posterior to the pelvic fins. A 32 mm PIT tag (RI-TRP-WR2B, half duplex, 134 kHz, diameter 3.85 mm and weight 0.8 g in air; Texas Instruments) was gently pushed anteriorly into the body cavity and the incision was left to heal without suture closure. Control fish were handled in the same manner as fish in the other treatments but no surgery was performed and no tag was implanted. Sham-operated fish were included in the experiment to isolate the effects of the surgery procedures from the effects of the PIT tags. The initial mean tag-to-body mass ratio in air was 5.1% (range: 2.2% to 13.6%) for Atlantic salmon tagged with 23 mm PIT tags and 6.7% (range: 2.7% to 14.8%) for those tagged with 32 mm PIT tags.

All control and sham-operated fish received a unique dye-mark combination on the left and/or right side of the caudal peduncle using a Panjet inoculator to allow for individual recognition during the experiment. Fish were marked with Alcian Blue, Irgafin Red P or a combination of the colors and the maximum number of dye-marks per fish was three. When used properly, jet injection of dye has no measurable effect on survival and growth of juvenile Atlantic salmon [31-33]. However, to ensure that the potential impacts of PIT tagging on mortality and growth were entirely isolated from any negative effects stemming from color marking, all PIT-tagged fish were also given dye-marks on the caudal peduncle region. Excess dye was carefully flushed off the fish with water as recommended by Hart and Pitcher [34]. The duration of the procedures for the control, sham-operated, 23 mm PIT tag, 23 mm PIT tag with suture closure, and 32 mm PIT tag treatment groups took on average 33, 38, 43, 77 and 44 s, respectively. Fish were not fed within 24 hours of surgery and the same surgeon performed all surgeries. All surgery equipment was disinfected (96% ethanol) prior to use and scalpel blades were changed frequently to avoid tearing of the tissue.

After treatment, fish were placed in an aerated barrel (60 L) until they recovered from anesthesia (3 to 4 min) and then transferred to the experimental tanks. The tanks (200 × 200 cm) were supplied with fresh water from a flow-through filtration system at a flow rate of 30 L min^{-1} ensuring well-oxygenated water. The water depth was adjusted to 35 cm, resulting in a water volume of 1,400 L. The average water temperature in the tanks was 6.9°C (range: 5.5°C to 7.5°C) and the photoperiod followed natural day-light cycles. Fish were fed during light hours with commercial trout pellets (Aller Performa, Aller Aqua, Denmark) at a ratio of 2% body mass per day using automatic feeders. The tanks were inspected daily for dead fish and rejected PIT tags. Dead fish were removed from the tanks, individually identified, measured, weighed, and examined externally and internally. The codes of rejected PIT tags found at the bottom of the tanks were recorded using a handheld reader (Agrident GmbH, APR350, Barsinghausen, Germany). After 35 days, the surviving Atlantic salmon were killed with an overdose of benzocaine, measured, weighed, and identified. The weights of fish tagged with 23 and 32 mm PIT tags were corrected by subtracting the weight of the tag from the final body mass (0.6 g and 0.8 g, respectively). The tagging incisions were inspected for infection, inflammation, and healing.

Data analysis

Fish that died or lost their PIT tag during the experiment were only used in analyses of survival and rates of tag retention. The percent tag loss was calculated as number of lost tags divided by the total number of fish tagged multiplied by 100. At the end of the experiment, the survival rate was calculated and a Chi-square test was used to compare survival among treatment groups. Tag-to-body mass ratio (%) in air was calculated with the formula:

$$\text{Tag–to–body mass ratio} = \left(\text{tag weight} * M_i^{-1}\right) * 100\%,$$

where M_i is the mass of fish prior to tagging.

The SGR (g day^{-1}) was calculated for each individual fish according to the following equation:

$$\text{SGR} = \left(\log_e M_f - \log_e M_i\right) * t^{-1},$$

where M_i and M_f are the initial and final mass (g), respectively, and t is the time in days. A one-way ANOVA was used to determine if there were differences in SGR among size classes of treatment groups. Tukey HSD multiple comparisons were subsequently performed to determine which treatment groups had significantly different SGR among size classes. Tank number was included as a random effect variable in the analyses.

An analysis of covariance was conducted to elucidate the effect of experimental treatments, length, and the interaction between these two variables on the body mass of the Atlantic salmon at the end of the experiment. The fish length-mass relationship was used as a proxy for body condition. Analyses were carried out on log-transformed length-mass data.

All statistical analyses were performed in SPSS 20.0 (Statistical Package for the Social Sciences; SPSS Inc, Chicago, IL, USA). Prior to analysis, data exploration was carried out as per Zuur *et al.* [35]. Assumption of homogeneity of variance and normal distribution for the models were ensured by visual inspection of residual plots. Variation in association with recorded mean values is given as standard error throughout. Statistical significance for all analyses was set at α = 0.05.

Abbreviations

ANOVA: Analysis of variance; DCV: Danish Center for Wild Salmon; FL: Fork length; HSD: Honestly significant difference; PIT: Passive integrated transponder; SGR: Specific growth rate.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MHL, ANT and KA designed the experiment. MHL and ANT carried out the experiment and analyzed the data. All authors contributed to draft the manuscript, and read and approved the final manuscript.

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